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Bacterial isolates and antimicrobial susceptibility in children with acute diarrhea at Ibn Sina Medical College, Bangladesh

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ABSTRACT

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Background: Infectious diarrhea is one of common cause of children diarrhea causing mortality and morbidity worldwide. This study was performed to identify the common bacteria and their antimicrobial susceptibility in children with diarrhea.

Methods: A retrospective study was conducted from April 2014 to March 2016 at IBN SINA Medical College Hospital, Bangladesh. Stool samples were cultured on MacConkey agar and blood agar. A standard biochemical procedure was used for full identification of bacterial isolates. Antimicrobial susceptibility tests were done on Mueller-Hinton agar by using disc diffusion method. Data were entered and analyzed by using SPSS version 20 and a p-value of <0.05 was considered statistically significant.

Results: A total of 186 stool samples were tested for bacterial isolation and 55 (29.57%) cases were found to have bacterial isolates. From the total bacterial isolates, the predominant isolate was *E. coli* 39 (70.91%) followed by *Salmonella* 9 (16.36%) and *Shigella* Spp.7 (12.73%). As much as 84.62% *E. coli* were resistant to co-trimoxazole and cefuroxime while 92.31% *E. coli* were sensitive to amikacin and 71.79% were sensitive to cefepime and gentamicin. *Salmonella* were 100% sensitive to cefepime, ceftriaxone, cefixime, ceftazidime, and ciprofloxacin. *Shigella* were 85.71% sensitive to amikacin and cefepime.

Conclusion: The results show that *E. coli* were the most frequently isolated pathogen in children. The majority of the bacterial isolates were resistant to multiple antibiotics. Hence, antibiotics susceptibility test is mandatory before prescribing any antibiotics.

Latar Belakang: Diare yang disebabkan oleh infeksi merupakan salah satu penyebab umum diare anak yang menyebabkan mortalitas dan morbiditas di seluruh dunia. Penelitian ini dilakukan untuk mengidentifikasi jenis bakteri dan kerentanan terhadap antimikroba pada anak dengan diare.

Tujuan Penelitian: Studi retrospektif dilakukan dari bulan April 2014 sampai Maret 2016 di IBN SINA Medical College Hospital, Bangladesh. Sampel tinja dikultur pada agar MacConkey dan agar darah. Prosedur biokimia standar yang digunakan untuk mengidentifikasi isolat bakteri. Tes kepekaan terhadap antimikroba dilakukan pada agar Mueller-Hinton dengan menggunakan metode difusi diskus. Data dianalisis dengan menggunakan perangkat lunak uji statistik SPSS versi 20 dan nilai $p < 0,05$ dianggap signifikan secara statistik.

Metode: studi retrospektif dilakukan dari bulan April 2014 sampai Maret 2016 di IBN SINA Medical College Hospital, Bangladesh. Sampel tinja dikultur pada agar MacConkey dan agar darah. Prosedur biokimia

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Hasil: Sebanyak 186 sampel tinja diuji untuk isolasi bakteri dan sebanyak 55 kasus (29,57%) ditemukan memiliki isolat bakteri. Dari keseluruhan isolat bakteri, didapatkan bahwa isolat bakteri yang dominan adalah *E.coli* 39 (70,91%) diikuti oleh *Salmonella* 9 (16,36%) dan *Shigella Spp.* 7 (12,73%). Sebanyak 84,62% *E.coli* resisten terhadap kotrimoksazol dan cefuroxime sedangkan 92,31% *E.coli* sensitif terhadap amikasin dan 71,79% sensitif terhadap sefepim dan gentamisin. Bakteri *Salmonella* sensitif terhadap sefepim, ceftriaxone, cefixime, ceftazidime dan ciprofloxacin (100%). *Shigella* sensitif terhadap amikasin dan cefepime (85,71%).

Kesimpulan: Hasil penelitian menunjukkan bahwa *E.coli* adalah patogen yang paling sering diisolasi pada anak-anak. Sebagian besar isolat bakteri resisten terhadap beberapa antibiotik. Oleh karena itu tes kerentanan terhadap antibiotik wajib dilakukan sebelum meresepkan antibiotik apapun.

INTRODUCTION

Infective diarrhea is one of the leading causes of morbidity and mortality among children under five years in the developing world and can be caused by a wide range of viruses, bacteria, or parasites.^{1,2,3} The prevalence of the different enteric pathogens varies with the geographical area.² Reports from different parts of the world have implicated various pathogens such as bacteria like; *Escherichia coli*, *Salmonella* species, *Klebsiella* species, *Enterobacter* species e.t.c. Parasites like; *Giardia lamblia*, *Entamoeba histolytica*, and viruses like the Rotavirus with the outbreak of infantile diarrheal disease.⁴⁻⁸ Antibiotic resistance is a major global public health concern, particularly in settings where few treatment options are available, either due to lack of availability or affordability of second line therapies.⁹ Commensal *Escherichia coli* can act as reservoirs of resistance genes in the human gut. These resistant genes might be rapidly transferred to other commensal

or pathogenic organisms.^{10,11} Faecal *E. coli* is regarded as a useful indicator of the spread of acquired antibiotic resistance genes in the community.^{12,13} Due to the overuse of antibiotics and change in epidemiology and antimicrobial resistance of bacterial agents, a study on the local epidemiology of bacterial diarrhea and antimicrobial susceptibility plays an important role in choosing the appropriate antibiotics for empirical treatment. Thus, this study was performed to identify common bacteria and their antimicrobial susceptibility in our region to support choosing a suitable antimicrobial agent.

METHODS

Study design, participants and data collection

A retrospective study was conducted from April 2014 to March 2016 at IBN SINA Medical College Hospital, Bangladesh. We reviewed the results of all stool cultures that presented bacterial growth that was taken from children between 0 and 15 years of age from April 2014 to March 2016. Data about patients' sex and age, pathogens isolated and their antimicrobial resistance patterns were taken from the Hospital Microbiology Laboratory unit registration books by using standard data collection format. During that period, 186 patients had their stools cultured and 55 samples presented bacterial growth.

Culture, identification and antimicrobial susceptibility testing

Stool specimens were collected using wide mouthed sterile plastic containers and transported to the Microbiology laboratory at IBN SINA Medical College Hospital within two hours of collection. Bacterial pathogens *E. coli*, *Salmonella* spp and *Shigella* spp were isolated and identified by conventional methods.¹⁴ Antimicrobial susceptibility testing was performed by disc diffusion method according to Clinical and Laboratory Standards Institute guidelines (CLSI) on Mueller-Hinton agar (Oxoid, England).^{15,16} Antibiotics tested were amikacin, amoxicillin, clavulanic acid, ciprofloxacin, gentamicin, cefepime, ceftriaxone,

co-trimoxazole, cefixime, ceftazidime and cefuroxime (Oxoid, England). According to the size of the zones of inhibition, the organisms were classified as sensitive, intermediate or resistant to a specific antibiotic according to CLSI guidelines.¹⁵ For the purpose of this study, intermediate sensitivity was considered as sensitive.

RESULTS

During the two-year period, the stools of 186 diarrheal patients aged 0 to 15 years were cultured and 55 (29.57%) samples presented bacterial growth and were included in the study. The characteristic of these patients, 103 were male (55.38%), while the other 83 were females (44.62%, Table 1).

Table 1. The result of culture from diarrheal stools in different sex.

Sex	Culture		Total N(%)
	Positive	Negative	
Female	20 (36.36)	63 (48.09)	83 (44.62)
Male	35 (63.64)	68 (51.91)	103 (55.38)
Total	55 (100)	131 (100)	186 (100)

$\chi^2 = 2.16$, $df = 1$, $P = 0.142$.

E. coli was the most frequent pathogen, being found in 39 cultures (70.91%), while *Salmonella* spp. was found in 9 cultures (16.36%) and *Shigella* spp. was found in 7 cultures (12.73%). The age distribution data revealed that out of 55 culture positive 23 cases (41.82%) were younger than 4 years, 6 cases (10.91%) were between five to nine years and 26 cases (47.27%) were between ten to fifteen years. *E. coli* is the most common cause of diarrhea in all age groups (Histogram 1). Among the *E. coli*, 84.62% were resistant to co-trimoxazole and cefuroxime. As much as 92.31% *E. coli* were sensitive to amikacin and 71.79% were sensitive to cefepime and gentamicin. Of these, 44.44% *Salmonella* were resistance to gentamicin and 100% *Salmonella* sensitive to cefepime, ceftriaxone, cefixime, ceftazidime, and ciprofloxacin. As much as 71.43% *Shigella* were resistant to amoxiclav, cefixime and gentamicin and 85.71% were sensitive to amikacin and cefepime (Table 2).

Table 2. Antimicrobial resistance pattern of bacterial isolates from stool samples participants

Antimicrobial agents	Resistant pattern of bacterial isolates (R %)		
	<i>E.coli</i> n (%)	<i>Salmonella</i> n (%)	<i>Shigella</i> n (%)
Amikacin	3 (7.69)	1 (11.11)	1 (14.29)
Amoxyclav	19 (48.72)	2 (22.22)	5 (71.43)
Cefepime	11 (28.21)	0 (0)	1 (14.28)
Ceftriaxone	27 (69.23)	0 (0)	2 (28.57)
Cifixime	28 (71.79)	0 (0)	5 (71.43)
Ceftazidime	31 (79.49)	0 (0)	3 (42.86)
Co-trimoxazole	33 (84.62)	1 (11.11)	3 (42.86)
Cefuroxime	33 (84.62)	1 (11.11)	4 (57.14)
Ciprofloxacin	20 (51.28)	0 (0)	2 (28.57)
Gentamycin	11 (28.21)	4 (44.44)	5 (71.43)

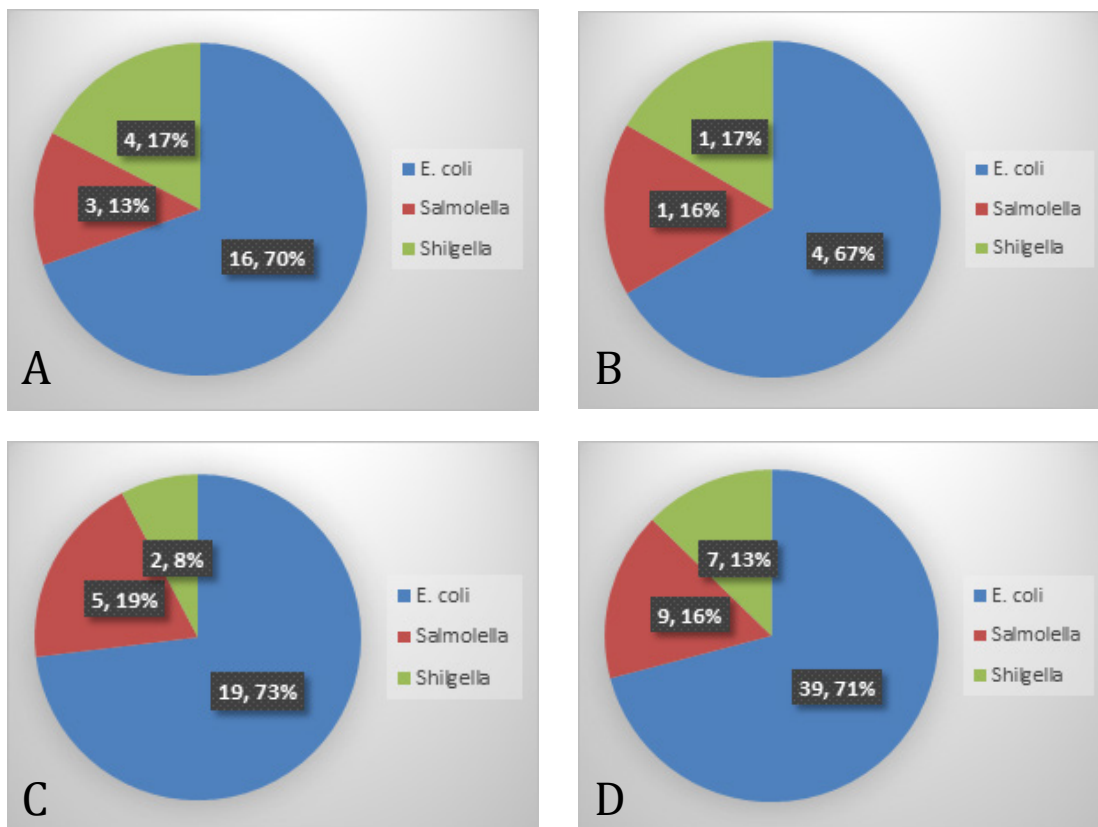


Figure 1. Distribution of the pathogens in diarrheal stools. A. distribution of pathogen in Up to years age, B. 5-9 years age, C. 10-15 years age, D. Total distribution of pathogen in child diarrheal stool

DISCUSSION

Given the importance of a careful characterization of the local epidemiology of diarrhea to guide specific antimicrobial therapy, we reviewed the results of 186 stool cultures from children aged 0 to 15 years old in which enteropathogens were detected. In this study, we found bacteria from more than a quarter of stool samples of children with acute gastroenteritis (29.57%). Our findings are in agreement with the results of other studies from the developing countries reported between 16.8 to 33%.¹⁶⁻¹⁷ We detected a higher incidence during the first year of life to four years of age (the period when children’s contact with environmental pathogens increases dramatically) and decreasing incidence levels in older children (5-9 years). In this study, the frequency of bacterial diarrhea in older age group (10-15 years) was significantly higher than the others that differ from the results of

the other studies.^{18,19}

E coli was the most common cause of bacterial diarrhea similar to many other previous studies.^{18,20-23} In our study, Salmonella (16.36%) is the second most common cause of diarrhea and Shigella was found in 12.73% cases. In other studies, Shigella was more frequent.²⁴⁻²⁵ Indeed, most patients with shigellosis require immediate the hospitalization.²⁶

In the present study, different bacterial species had different level of resistance pattern to different antibiotics. However, E. coli isolates were found to have a high rate of resistance to cotrimoxazole (84.62%), cefotaxime (87.18%) and cefixime (71.79%). All in all, our susceptibility patterns are slightly more favorable than those reported by the large multicenter SENTRY Antimicrobial Surveillance Program Report for Brazil and Latin America.²⁷

We found that the Salmonella strains were

multi-susceptible, as high rates of resistance were not found against any of the drugs. We found no resistant to ciprofloxacin. But resistance to ciprofloxacin among non typhoidal *Salmonella* strains isolated from stool specimens has already been reported from several parts of the world.^{28,29} On the other hand, a multicenter Latin American study published by Gales et al. did not isolate any strain of *Salmonella* resistant to fluoroquinolones from blood samples.³⁰ However, *Salmonella* spp. strains isolated from blood samples are recognized to be more susceptible than those from stools.³¹ Furthermore, the fluoroquinolone tested by Gales et al. was gatifloxacin, a new 8-methoxyfluoroquinolone that is supposed to be active against most ciprofloxacin-resistant bacteria.

Separate assessment of antimicrobial resistance patterns indicates that *Shigella* is more difficult to treat and requires careful consideration at the choice of antimicrobial therapy. However, *Shigella* resistance has not reached the alarming multi-resistance rates reported in other countries, where resistance to ciprofloxacin and to ceftriaxone is a reality.³²⁻³⁴ While we detected high resistance rates to amoxiclav, gentamicin and cefixime (71.43%). And 71.43% were sensitive to ceftriaxone and ciprofloxacin. Prescription of antibiotics without laboratory guidance and over sales of antibiotics without proper drug prescription may be some of the different factors that can contribute to this high-level drug resistant pattern.

CONCLUSION

In conclusion, the results show that *E. coli* was the most frequently isolated pathogen in children. The majority of the bacterial isolates were resistant to multiple antibiotics. Hence, antibiotics susceptibility test is mandatory before prescribing any antibiotics. We recommend that antibiotics susceptibility test is mandatory before prescribing any antibiotics in children diarrhea.

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COMPETING INTEREST

The author declared that no competing interest with respect to the authorship and/or publication of this research paper

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